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Investigation on pH dependent uptake of Cr(III) and Cr(VI) by Baker's yeast

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Yeast cells of *Saccharomyces cerevisiae* were found to accumulate ^{51}Cr(III) radioisotope at basic *p*H in trace level. There was no uptake of Cr(VI) at the same *p*H (~10.5). An assay of the products of cell lysis reveals that Cr(III) first gets adsorbed at the cell wall and then slowly enters the cytoplasm. On the other hand Cr(VI) has a faster penetration into the cytoplasm which increases with time, attains a maximum value and then release the metal ion from cytoplasm. The behavior and uptake kinetics of ^{51}Cr(III) or ^{51}Cr(VI) were studied using γ-spectrometry.

Keywords: *Saccharomyces cervisiae,* Baker's yeast, Cr (III) and Cr(VI) uptake

Metal-microbe interactions have gained growing interest in recent years. Development of cost effective technologies, using biological reagents for desired chemical processes have become an intensive area of exploitation over the past decade. Among the promising biosorbents for different metal species, *Saccharomyces cerevisiae* is unique compared to other fungi as it is widely used in food and beverage production, easily cultivated using cheap media and also a byproduct of fermentation industry. Earlier, different strains of *Saccharomyces cerevisiae* have been used for metal uptake studies for different elements like, Cd, Cu, As, etc[1-3]. Yeast cells were utilized for trace enrichment of metal ions from industrial waste, dam water and sewage plant, which have direct consequences of bio-magnifications of these metals through the food chain[4]. Even precious metals and light element like aluminium were recovered using cells of Baker's yeast[5,6]. Most interestingly, *S. cerevisiae* can distinguish between different species of same metal based on their toxicity like Sb(III) or Sb(V), organic and inorganic mercury, etc[7]. This property makes *S. cerevisiae* useful not only for the bioremediation, removal or recovery of metal ions, but also for analytical measurements. However, the mechanism behind these processes of the biosorption is disputable and remains to be further elucidated.

Earlier, chromium uptake and bioremediation by yeast were also studied extensively[8]. Hexavalent chromium is more toxic than Cr(III) because its oxidizing potential is high and it easily penetrates biological membranes, can cause damages to DNA and other tissue structures. On the other hand in lower dose Cr(III) is considered as an essential trace element for the proper functioning of living organisms. Cr(III) is an essential nutrient that enhances insulin action and thus influences carbohydrate, lipid and protein metabolism[9,10]. A variety of yeast genera were taken to study the Cr uptake capacity using cell culture method, and in the presence of growth-inhibitory chromium concentrations[11]. The cellular Cr content in the yeast *Pichia guilliermondii* ranged from 1.2-4.0 mg/g d.w. and 0.4-0.9 mg/g d.w., for Cr(III) and Cr(VI) forms. It has been found that treatment of Cr(VI) with *P. guilliermondii* triggers over-synthesis of riboflavin and on the other hand addition of exogenous riboflavin increases *P. guilliermondii* resistance to both Cr(III) and Cr(VI)[12].

However, despite of the wide study on Cr bioaccumulation by different yeast genera, till date no report has been made on the *p*H dependent differential behaviour of Baker's yeast towards Cr(III) and Cr(VI). Moreover, the accumulation kinetics of chromium depending on its species in yeast cells has never been reported. In this work the behaviour of yeast cells towards Cr(III) and Cr(VI) at different *p*H and time intervals has been reported.

Experimental Procedure

The radiotracer ^{51}Cr was obtained by irradiating Cr(VI) in the form of $K_2Cr_2O_7$ and Cr(III) in the form of $Cr(NO_3)_3.9H_2O$ with thermal neutron flux of 10^{11}n/cm^2/s for 8 h at CIRUS reactor, Mumbai, India resulting in specific activities 0.043 mCi/g and 0.016 mCi/g respectively. The irradiated chromium salts were dissolved in measured volume of triple distilled water. The concentration of the resulting stock solutions were 0.038 M and 0.056 M for $K_2{}^{51}Cr_2O_7$ and $^{51}Cr(NO_3)_3$, respectively. Yeast extract (HI Media, India), bactopeptone (HI Media, India), agar and dextrose (SISCO Research Laboratories, India) were used for the preparation of media. The extent of bioaccumulation of

^{51}Cr by yeast was measured by a well type HPGe detector having a resolution of 2.0 keV at 1.33 MeV.

Two different strains of yeast, AP22 (*MAT*α *leu2 his3 trp1 ura3*) having a background of G. R. Finks laboratory and 699 (*MAT*a *leu2 his3 ura3 trp1 ade2*) having W303 strain background were taken for bioaccumulation studies. The yeast cells were grown on solid medium containing, yeast extract, 0.5; bactopeptone, 1; dextrose, 1 and agar, 1.5%. The yeast cells were suspended in 5 mL water and shaken to make a uniform mixture. For the study of the variation in metal uptake with *p*H, 3 mL of the solutions of required *p*H, adjusted with dil. HCl (0.01 M) or NH_3 (10%) solution, were pre-equilibrated with 100 µL of yeast suspension for one hour. Stock solutions containing 100 µL of ^{51}Cr(III) or ^{51}Cr(VI) were added to this system and shaken for 10 min. The activity of ^{51}Cr was measured (C_1) for 10 min in a fixed geometry before mixing with yeast suspension. The resulting mixture was then filtered using a syringe filter (0.45 µm pore diameter) and the filtrate was taken for counting (C_2). All the results were compared with the count of the isotopes before mixing. The accumulation (%) by yeast cells has been calculated using the formula

$$A=(1- C_2/C_1)\times 100\%$$

The number of yeast cells present per mL of the experimental set was measured using a haemocytometer and was found to be 7×10^{12} in average. To determine the rate of accumulation with time, yeast cells were allowed to remain in contact with the solution containing ^{51}Cr(III) or ^{51}Cr(VI) for different intervals of time. The *p*H was adjusted at which maximum accumulation was shown from the previous results. The resulting solutions were filtered after varying intervals of time and the filtrate were taken for γ-spectrometric studies.

In a separate experiment chromium accumulated yeast cells were washed thoroughly with 0.5 M phosphate buffer solution and then vortexed with half-volume glass beads in the same buffer medium for mechanically disrupting the cells[13]. The broken cells along with the glass beads were then centrifuged to separate the cell debris at the bottom and the supernatant containing the cytoplasm. Both the supernatant and the residue were separately taken for γ-spectrometric study to determine the accumulation site of chromium in the cell. All the experiments were repeated at least thrice to check the reproducibility of results.

Results and Discussion

It has been observed that the accumulation patterns of Cr(III) and Cr(VI) by the yeast cells differ widely but there is no significant change in the uptake pattern of the two strains AP22 and 699. After 10 min shaking of yeast cells with chromium salts, it has been observed that the accumulation of Cr(III) increases with *p*H and at *p*H 10.5 quantitative extraction is possible. Under the same condition accumulation of Cr(VI) is negligible throughout the entire *p*H range with a maximum of 25% at *p*H 3 and becomes zero at *p*H 10.5 (Figs 1 and 2).

The uptake kinetic study of Cr(III) by the yeast cells reveal that uptake is very high at the beginning with a minima at 1 h and attains the maximum value after about 3 h and then remains constant for several hours (Fig. 3). The uptake of Cr(VI) though in much lesser amount when compared with Cr(III) follows different pattern. The uptake of Cr(VI) slowly

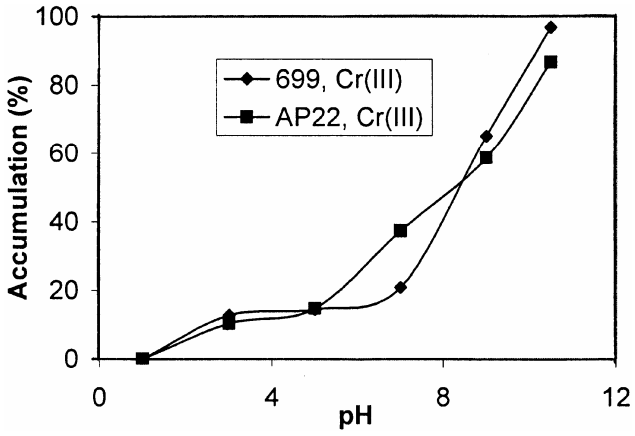

Fig. 1— Extraction profile of Cr(III) uptake with variation of *p*H.

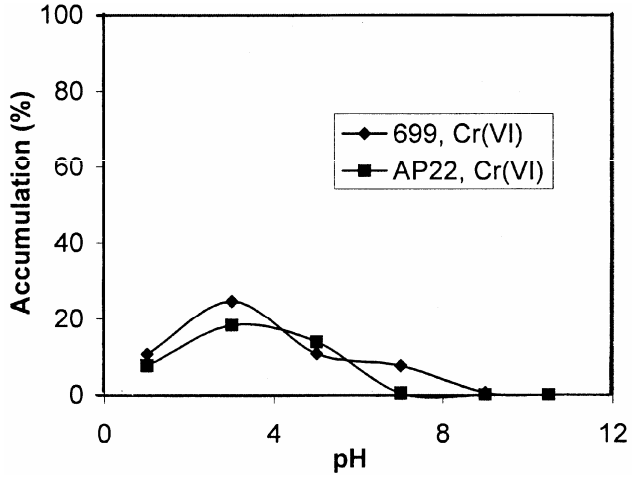

Fig. 2— Extraction profile of Cr(VI) uptake with variation of *p*H

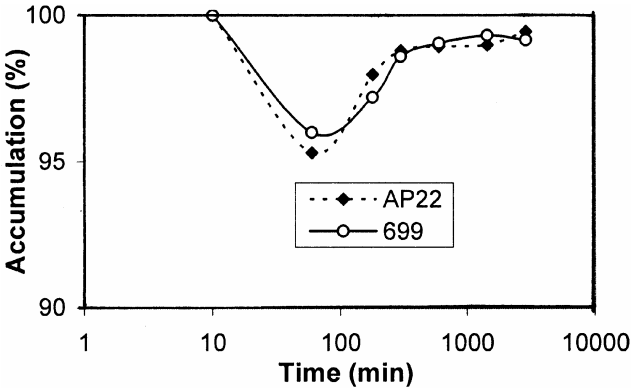

Fig. 3— Extraction profile of Cr(III) with time.

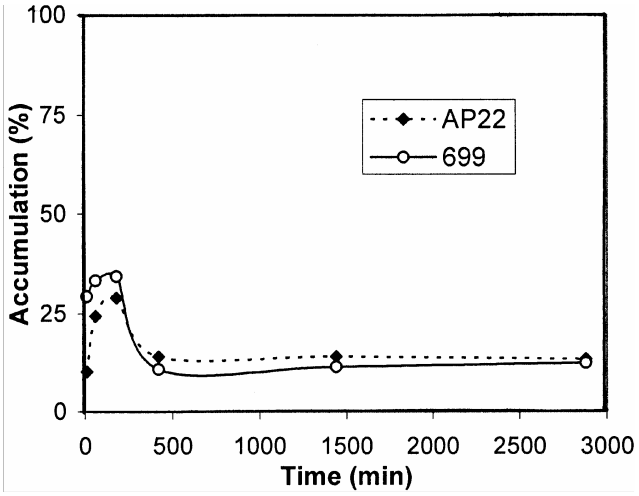

Fig. 4— Extraction profile of Cr(VI) with time.

increases upto three hours and then the accumulated Cr(VI) gets released from yeast cells. Finally, after about 48 h only a small fraction (10-12%) remains inside the cell (Fig. 4).

To know the destination of chromium species, the ^{51}Cr accumulated yeast cells were broken as described in the experimental section. Lysis products show that after three hours of incubation, Cr(III) remains completely in the residue portion. This implies that Cr(III) was accumulated at the cell wall and could not penetrate into the cytoplasm. On the other hand Cr(VI) was found in the supernatant portion which is nothing but the cytoplasm. This implies that Cr(VI) has a faster kinetics to enter the cytoplasm. A similar study after 24 h accumulation revealed that both Cr(III) and Cr(VI) were present in the cytoplasm and not in the cell debri. This is probably due to the fact that Cr(III) remains as $Cr(OH)_4^-$ in solution state at *p*H 10.5 and therefore initially binds with cationic part of the cell wall; afterwards slowly migrates into the cell possibly through interactions with polysaccharides, glucoprotein and soluble peptides present at the extracellular surface[14]. The results were found to be reproducible when repeated in a similar fashion.

The developed method is simple, nontoxic and environmentally sustainable for removal of Cr(III) quantitatively from basic medium. The method is also efficient to differentiate between essential Cr(III) and toxic Cr(VI) species of chromium. Cr(III) is accumulated in the cytoplasm of yeast cell; while Cr(VI) though penetrates into the cytoplasm but also gets rejected from the cell after a longer time.

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